

WEST Search History

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DB=USPT,JPAB,EPAB,DWPI,TDBD; PLUR=YES; OP=OR

L2 L1 and liposome\$

67 L2

L1 enzyme\$ same (together or along) same stabiliz\$

581 L1

END OF SEARCH HISTORY

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L2: Entry 31 of 67

File: USPT

Sep 25, 2001

DOCUMENT-IDENTIFIER: US 6294365 B1

TITLE: Method and formulation for stabilization of enzymes

Brief Summary Text (12):

The use of sugars such as the disaccharide trehalose as stabilizing agents has been used in conjunction with lyophilization to stabilize polysaccharides (Guthohrlein and Helting, European Patent No. GB 2 009 198 A) and liposomes (Crowe et al., 1987). Lyophilization has also been used to stabilize tumor necrosis factor in the presence of a nonionic surfactant and sugars such as trehalose (Hayashi and Komiya, European Patent No. GB 2 126 588 A). Enzymes that have been stabilized with sugars during lyophilization include phosphofructokinase (Carpenter et al., 1987), which was stabilized with glucose, galactose, maltose, sucrose, and trehalose, and alkaline phosphatase (Ford and Dawson, 1992) which was stabilized with mannitol, lactose, and trehalose.

Detailed Description Text (50):

A mixture of the three enzymes was made with the stabilizing formulation of BSA, maltitol, trehalose, dextran, and Universal buffer. The deglycerolized enzymes were added in the ratio of 1.6 units of AMV-RT, 144 units of T7 RNA Polymerase, and 0.48 units of RNase H per reaction (adjusted for 20 .mu.l reaction format), with the total enzyme volume being about 15% of the total mixture volume. BSA was added to a final concentration of 3 mg/ml (w/v), maltitol to a final concentration of 10.5% (w/v), trehalose to a final concentration of 10% (w/v), Dextran T-500 to a final concentration of 2.4%, and Universal buffer to volume. The wet enzyme mixture consisted of 2 units of AMV-RT, 180 units of T7 RNA Polymerase, and 0.6 units of RNase H, along with 2.5.mu.g BSA per reaction. The deglycerolized mixture was quick-frozen and lyophilized. After reconstitution, it showed the same ability to amplify 10.sup.3, 10.sup.2, and 10.sup.1 input molecules of target RNA per reaction as a wet enzyme mixture (see FIG. 2). Lane 1 is a no target (NT) or negative control. Lane 2 is a detection, or gel control, of a reaction that gave strong signal with input of 10.sup.1 target molecules. Lanes 3-6 are replicate reactions using 10.sup.3 target molecules input. Lanes 7-10 are replicate reactions using 10.sup.2 target molecules input. Lanes 11-14 are replicate reactions using 10.sup.1 target input, showing strong signal for 3 of 4 reactions. (Note that to those skilled in the art, it is expected that input of 10.sup.1 copies of HIV-1 RNA in NASBA.RTM. yields a 50% probability of detecting positive reaction results.) Assessment was made qualitatively, by scoring of band intensity on ELGA gels. Therefore, the ability of this enzyme mix to function in NASBA.RTM. was not impaired after lyophilization and subsequent reconstitution. As a result of these findings, all subsequent stability experiments used samples from this lyophilization run and 10.sup.2 input copies of HIV-1 RNA.

Detailed Description Text (63):

Crowe, J. H., B. J. Soargo, and L. M. Crowe. Preservation of dry liposomes does not require retention of residual water. Proc. Natl. Acad. Sci., 84: 1537-1540 (1987).

Other Reference Publication (5):

Crowe et al., "Preservation of Dry Liposomes Does Not Require Retention of Residual Water," Proc. Natl. Acad. Sci., 84:1537-1540 (Mar., 1987).

WEST

Generate Collection

Print

L2: Entry 51 of 67

File: USPT

Mar 2, 1999

DOCUMENT-IDENTIFIER: US 5876992 A

TITLE: Method and formulation for stabilization of enzymes

Brief Summary Text (12):

The use of sugars such as the disaccharide trehalose as stabilizing agents has been used in conjunction with lyophilization to stabilize polysaccharides (Guthohrlein and Helting, European Patent No. GB 2 009 198 A) and liposomes (Crowe et al., 1987). Lyophilization has also been used to stabilize tumor necrosis factor in the presence of a nonionic surfactant and sugars such as trehalose (Hayashi and Komiya, European Patent No. GB 2 126 588 A). Enzymes that have been stabilized with sugars during lyophilization include phosphofructokinase (Carpenter et al., 1987), which was stabilized with glucose, galactose, maltose, sucrose, and trehalose, and alkaline phosphatase (Ford and Dawson, 1992) which was stabilized with mannitol, lactose, and trehalose.

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Other Reference Publication (5):

Crowe et al., "Preservation of Dry Liposomes Does Not Require Retention of Residual Water" Proc. Natl. Acad. Sci., 84:1537-1540 (Mar. 1987).

WEST**End of Result Set**

Generate Collection

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L2: Entry 67 of 67

File: USPT

May 14, 1991

DOCUMENT-IDENTIFIER: US 5015483 A

TITLE: Liposome composition for the stabilization of oxidizable substancesAbstract Text (1):

A stable, food-compatible liposome is prepared by dissolving a lipophilic material in a phospholipid followed by the addition of water or an aqueous solution and mixing by sonicating to producing a liposome having the lipophilic material encapsulated in the lipid bilayer. The preferred lipophilic materials include any readily oxidizable lipid and in particular the omega-3 fatty acid containing fish oils. Other lipophilic materials which can be encapsulated in the lipidic bilayer include flavorants, acidulants, preservatives and antioxidants. The resulting liposomes provide a stabilizing vehicle for the lipophilic materials to reduce the occurrence of oxidation and rancidity. Liposomes prepared according to the disclosed method exhibit none of the unpleasant odor and flavor characteristics normally associated with oxidized or rancid oils. The liposomes provide an extended shelf life of the unstable oils and can be added directly to the food product as a dispersion during the manufacturing stage or dried to a free flowing powder for later use.

Brief Summary Text (3):

The present invention relates to the production of liposomes containing lipophilic materials dissolved in the lipid layer of the liposome. More specifically, the invention is directed to the stabilization of oxidizable unsaturated lipophilic compounds by the encapsulation of the material in the lipidic layer of a liposome to retard or inhibit oxidation. The invention further relates to a lipophilic material stabilized by encapsulating the material in a liposome having a predetermined thermal transition temperature and shear resistance to withstand the physical treatments normally encountered in commercial food preparation. The liposomes can be used to introduce a readily oxidizable lipidic component to foods in a pure form. The liposomes offer a means whereby the lipophilic component can be easily dispersed in an aqueous phase. The liposomes can also be introduced to food compositions to slowly release the material dissolved in the lipidic layer over an extended period of time.

Brief Summary Text (5):

Liposomes have been primarily used in recent years as delivery and carrier systems by encapsulating various compounds in the aqueous layer of the liposome. The emphasis of liposome development has been in the delivery of bioactive compounds. In the field of pharmaceutical and drug delivery, liposomes encapsulating drugs in the aqueous layer have been used successfully by suspending the liposome in a nonreactive carrier which can be injected directly into the treatment site to deliver an effective amount of the encapsulated drug.

Brief Summary Text (6):

Liposomes are essentially closed bilayer membranes in the form of vesicles or sacs containing an entrapped aqueous phase. Liposomes having a single bilayer membrane are referred to as unilamellar vesicles, whereas liposomes having a number of concentric lipid bilayers separated by an aqueous phase are referred to as multilamellar vesicles. Recently, the attention on liposome preparation has focused on the liposomes with several bilayers, since these multilamellar vesicles demonstrate possibilities of encapsulating larger amounts of components than in the unilamellar vesicles

Brief Summary Text (7):

Liposome preparation methods have been known for sometime. One of the earlier methods of preparing liposomes is described by Bangham et al. in J. Mol. Bio. Vol.13 pp. 238-252 (1965). According to that method, a phospholipid, such as phosphatidylcholine, is suspended in an organic solvent which is then evaporated to dryness, resulting in a waxy phospholipid deposit on the wall of the vessel. An aqueous phase containing a desired encapsulant is added to the reaction vessel, and mechanical means, such as ultrasonics, are used to disperse the resulting liposomes. The liposomes produced by this method are predominantly unilamellar vesicles (ULV). The liposomes have a lipidic bilayer, where the non-polar hydrophobic ends of the phospholipid orient themselves toward the center of the liposome bilayer, and the polar hydrophilic ends orient themselves towards the aqueous layer. Liposomes prepared according to this method are generally quite small, which restricts the ability to encapsulate large macromolecules.

Brief Summary Text (8):

As a consequence of these limitations, efforts have been made to increase the volume of the entrapped material. These methods have included the formation of inverse micelles or liposome precursors. The precursors are generally vesicles containing an aqueous phase surrounded by a monolayer of lipid molecules oriented so that the polar head groups are directed towards the aqueous phase. Liposome precursors are formed by adding the aqueous solution to be entrapped to a solution of an amphiphilic lipid in an organic solvent and sonicating the mixture. The organic phase is evaporated in the presence of the liposome precursors which associate with the excess lipid. The resulting liposomes are then dispersed in an aqueous phase. An example of such a process is disclosed in U.S. Pat. No. 4,224,179.

Brief Summary Text (10):

In general, the larger a liposome, and the fewer layers it has, the more liquid it can encapsulate. Since the carrier liquid in which a liposome is dispersed can differ compositionally from that contained within the liposome itself, liposomes are potentially useful as biodegradable, relatively nontoxic vehicles for administering a component without risk of prematurely degrading the component, as might occur, for example, upon exposure to air or contact in the gastrointestinal tract.

Brief Summary Text (11):

Liposomes prepared from naturally occurring phospholipids have a tendency to be unstable during storage, thereby limiting their utility. This inherent instability results in a breakdown of the bilayers and the entrapped materials of the liposome diffusing into the surrounding liquid medium. This dispersing of the released materials can result in the contamination of the remaining liposomes by permeation of the materials from the surrounding medium into the liposome itself. The permeability characteristics of a liposome membrane bilayer can be altered by the addition of different stabilizers. For example, cholesterol can be mixed with other liposome-forming amphiphilic lipids to enhance the orientation of the amphiphilic lipids into a more orderly crystalline array. The more orderly arrangement stabilizes the bilayer and reduces permeation of materials.

Brief Summary Text (12):

A major use of liposomes to date has been in the medical field, by employing liposomes which entrap an effective amount of a water-soluble pharmaceutical compound and suspending the liposome in a pharmaceutically acceptable carrier. The advantages of using liposomes for this purpose arise from the fact that, when liposomes are injected into animals, they are taken up rapidly by the cells and intra-cellular lysosomes. Liposomes can be made to be relatively impermeable, and since they are rapidly taken up by the cells and removed from the circulatory system, the pharmaceutical remains concentrated in the liposome and unexposed to the plasma until such time as the liposomes are broken. This technology and knowledge primarily developed for the medical field offer alternative uses of liposomes, outside the delivery of pharmaceuticals, which have not been fully explored or utilized.

Brief Summary Text (13):

Liposomes may be prepared from a wide variety of lipidic compounds, including alkylamines, gangliosides, cardiolipin, and phospholipids. Some of the more widely used lipids include the phospholipids wherein the phosphatidyl portion contains ester

groups, for example C.sub.14 to C.sub.20, saturated or unsaturated, fatty acids. Specifically, liposomes may be prepared from any one or combination of dicetylphosphate, distearoylphosphatidic acid, dipalmitoylphosphatidylcholine, dimyristoylphosphatidylinositol, mono and dialkyl esters of phosphatidylserine, and dioleoylphosphatidylglycerol. In addition, lysophosphatides, wherein the fatty acid ester of C.sub.1 or C.sub.2 has been removed, can be mixed with the above-noted lipids to form liposomes. Other synthetic phosphatidyl compounds used include those wherein a sulfonium, phosphonium, or quaternary ammonium polar head moiety has been modified by the addition of hydrocarbon groups, particularly alkyl groups.

Brief Summary Text (14):

In the preparation and processing of food products, it is desirable to include various components or additives such as enzymes, vitamins, preservatives, acidulants or edible oils. Some of these additives can be combined in foods only in limited amounts due to their unpleasant flavor characteristics. In addition, some desirable additives, such as some vegetable oils and fish oils, are readily oxidizable and develop an unpleasant odor and flavor after a relatively short period of time. Some additives simply degrade over time or after exposure to oxygen and light and become ineffective. As a result of these inherent adverse characteristics, efforts have been made to modify the additives in some manner to disguise the flavor or stabilize the components. These efforts have not employed the formation of food-compatible liposomes which contain readily oxidizable substances in the lipidic bilayer.

Brief Summary Text (15):

One example of the prior art liposomes is U.S. Pat. No. 4,508,703, which discloses that a partially or totally hydrophobic substance may be added into the lipidic bilayer of liposomes or hydrated lamellar phases. The hydrated lipidic lamellar phases are prepared from a pulverulent mixture for producing liposomes which are primarily intended to be used in the release and delivery of drugs, as well as in the cosmetic and food industries. A mixture of amphiphilic and hydrophobic lipid constituents is produced in an organic solvent and atomized to produce a dry pulverulent material. Suitable amphiphilic lipids include phospholipids, glycolipids or phosphoaminolipids, such as egg or soya lecithin, a phosphatidylserine, a cerebroside or a sphingomyelin. The hydrophobic or partially hydrophobic constituents include sterols or esters thereof, such as cholesterol, aliphatic fatty acids or esters, aliphatic fatty amines, fatty acid acylated amides, polypeptides, vitamins or extracts of animal or vegetable origin. These hydrophobic constituents are added to the amphiphilic lipid to improve the physical characteristics of the resulting liposome, such as wall strength, permeability and stability. The liposomes and hydrated lipidic lamellar phases are produced by dispersing the pulverulent mixture in a suitable aqueous medium. The aqueous medium may contain water-soluble substances, such as proteins for use in foods or sodium carboxylate pyrrolidone for use in cosmetics.

Brief Summary Text (19):

"Accelerated ripening of cheese using liposome-encapsulated enzyme" Kirby et al. International Journal of Food Science and Technology Vol. 22, pp.355-75 (1987) discloses the use of liposomes, to encapsulate an enzyme in the aqueous phase, for accelerating the ripening of cheese. Encapsulating the enzyme within the liposomes stabilizes the enzyme, such that it remains active longer. This produces a 100-fold increase in rate over conventional methods of ripening cheese. The liposomes are prepared by the dehydration/rehydration vesicle procedure (DRV), using egg phosphatidylcholine and soya lecithin dissolved in chloroform:methanol. The solution is dried by rotary evaporation to form a thin film of the lipid around the inside of the flask. The lipid is dispersed by swirling with distilled water to produce an opalescent dispersion. Neutrase dissolved in distilled water is then added to the dispersion, and the dispersion is frozen by swirling in a liquid nitrogen bath. The frozen dispersion of liposomes is then freeze-dried. DRV's were formed by adding distilled water to give a complete suspension of the material.

Brief Summary Text (29):

The present invention is therefore directed to a food-compatible liposome composition containing highly oxidizable lipophilic materials dissolved in the lipid layer of a liposome. The liposomes prepared according to the invention provide a means to prevent or inhibit oxidation of the encapsulated lipophilic material. The invention

is further directed to a suitable lipid-containing food product containing large amount of the novel liposome composition but free from adverse effects on its flavor and odor.

Brief Summary Text (31):

The disadvantages and limitations of the prior art are obviated by providing an effective and efficient means of stabilizing readily oxidizable lipophilic materials. The present invention is particularly directed to the stabilization of readily oxidizable, labile lipophilic components, such as fish oils, by encapsulation of the components in the lipidic bilayer of liposomes. The resulting liposomes provide an excellent carrier for the lipophilic materials by placing them in intimate contact with oxygen-stable saturated fatty esters of the phosphatide. The lipid layer of the liposomes further provides an oxygen barrier to inhibit propagation of free-radical chain reactions.

Brief Summary Text (32):

More particularly, the invention is directed to a method of effectively stabilizing readily oxidizable lipids. The liposomes prepared according to the invention provide a stable structure to encapsulate reactive and oxidizable lipids and to isolate them from the surrounding environment. The liposomes further provide a convenient and effective means for producing a stabilized lipid composition and a vehicle which can be readily dispersed in an aqueous medium or in a lipid medium. By encapsulating the oxidizable lipidic component in the lipid bilayer, the component does not come into direct contact with the aqueous surroundings, which reduces the risk of unwanted reactions for extended periods of time.

Brief Summary Text (33):

In the preferred embodiment of the invention, the encapsulated lipophilic material employed is an unsaturated triglyceride omega-3 fatty acid-containing fish oil which is deodorized and refined. The omega-3 fatty acid fish oils are dispersed in the phospholipid bilayer of the liposome to obtain a concentration of about 30% by total weight of the lipids in the system. Other unsaturated lipophilic components can include eicosatetraenoic acid, eicosapentaenoic acid, docosahexaenoic acid, oleic acid, linoleic acid, linolenic acid, and arachidonic acid.

Brief Summary Text (35):

The liposomes prepared according to the invention may be added directly to food preparations containing a high fat content, such as margarine, compound coatings, and other water-in-oil emulsions. The liposomes are readily dispersible in an aqueous phase or in an emulsion where a continuous aqueous phase is present, such as mayonnaise and other oil-in-water emulsions. The liposomes, when added to such compositions, are stable over extended periods of time and are resistant to moderate temperature changes. The liposomes may further be applied as a coating composition to food products to provide, for example, a flavoring or coloring agent. The liposomes are particularly suitable for dispersion in an aqueous system in which the entrapped lipophilic component would otherwise result in an undesirable phase separation. In this manner, it is possible to disperse the water-insoluble lipophilic component into an aqueous solution or aqueous phase.

Brief Summary Text (36):

According to the preferred embodiment of the invention, the liposomes are REVs prepared according to conventional practices. The method employed follows that disclosed in U.S. Pat. No. 4,235,871 by Szoka Jr. and Papahadjopoulos. The phospholipid, such as phosphatidylcholine and/or cholesterol, and the lipophilic material to be encapsulated are dissolved in an organic solvent, which is then evaporated in a flask by rotary evaporation. The system is then purged with nitrogen and the lipid redissolved in an organic solvent. The aqueous phase is then added, and the system is kept under nitrogen while being sonicated for 2-5 minutes in a bath-type sonicator until a homogeneous opalescent dispersion appears. The dispersion is placed in a rotary evaporator to remove the excess organic phase until a thickened gel-like mass is obtained. The method according to the invention has produced satisfactory liposomes containing up to 10% by total weight (i.e. liposomes and the residual water surrounding the liposomes) of the encapsulated lipophilic component. This corresponds to about 30% by total weight of the lipids present.

Drawing Description Text (3):

FIG. 1 is a graph comparing the peroxide values, expressed in milliequivalents per kilogram of lipid, over a period of 90 days for unencapsulated fish oil, fish oil encapsulated in liposomes made from soybean phosphatidylcholine, and fish oil encapsulated in liposomes made from hydrogenated phosphatidylcholine.

Detailed Description Text (3):

The method according to the invention provides a usable composition which remedies these adverse characteristics of oxidizable lipophilic materials. In one embodiment of the invention, the liposomes may be in a dry powder form or as an aqueous dispersion, as discussed hereinafter in greater detail. As used throughout this disclosure, the term lipophilic component is intended to refer to a component which is soluble or miscible in the lipidic bilayer of a liposome and is the component of particular interest to be stabilized by being encapsulated in and carried by the liposomes. The solubility of the lipophilic component is dependent on several factors, including chain length, unsaturation, melting point and the like. The solubility of the lipophilic material will generally increase as the chemical and physical properties of the phospholipid and the lipophilic components become more alike. For example, the long-chain, highly unsaturated fish oils are generally more soluble in the phospholipids containing up to 60-70% linoleic acid and 6-13% oleic fatty acid esters. The triglycerides are generally more soluble in the phospholipids than the fatty acids and are therefore preferred.

Detailed Description Text (4):

Liposomes of the invention may be prepared by any of the conventional techniques, such as dehydration/rehydration and reverse phase evaporation (REV). The preferred method of the invention is by REV, since the vesicles generally contain a larger volume of entrapped materials. The lipophilic components, before encapsulation, are preferably purified and deodorized as necessary using standard known procedures.

Detailed Description Text (5):

The REV's according to the invention are prepared generally by the method disclosed in U.S. Pat. No. 4,235,871, which is hereby incorporated by reference, and Szoka et al., "Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation", Proc. Natl. Acad. Sci. U.S.A. Vol. 75, pp. 4194-4198 (1978).

Detailed Description Text (6):

The liposome-forming polar lipid containing the dissolved lipophilic component is dispersed in an organic solvent. The solvent is then evaporated under vacuum to produce a lipid film on the wall of the vessel. After redissolving the lipid in an organic solvent, an aqueous medium is then added to the vessel and mixed with the lipid to produce a liposome suspension. Mixing occurs by such means as homogenizing or sonicating at standard frequencies and time periods. The liposome dispersion according to the present invention can then be stored as a dispersion or dried by freeze drying to remove the excess water. The liposome powder can be stored and later rehydrated as desired and added directly to the other ingredients of the finished composition, as discussed hereafter in greater detail. The liposomes have the distinct advantages of being quite stable and providing an effective stabilizing means for reactive and oxidizable lipophilic materials. The liposomes can be easily dispersed in an aqueous medium, regardless of whether the liposomes are stored as an aqueous dispersion or as a dried powder.

Detailed Description Text (7):

In the preferred embodiment of the invention, the lipophilic material is a lipid oil containing a high degree of unsaturation. Since autoxidation of unsaturated fats and oils involves a chain reaction of the double bonds, it is desirable to have the lipidic bilayer of the liposome formed from a saturated phospholipid. It is believed that the saturated lipidic layer tends to "dilute" the unsaturation of the dissolved or encapsulated component. The dilution reduces the likelihood of formation of free radicals and reduces the number of available reaction sites for any radicals which do form. The oxidizable materials in the lipidic bilayer are isolated from the environment, thereby reducing the likelihood of exposure of the oxidizable material to oxygen. Since the encapsulated highly oxidizable lipophilic component is dispersed throughout the comparatively non-reactive lipidic bilayer of the discrete liposomes,

propagation of the free-radical chain reaction from one unsaturated chain to another is inhibited, as is also the propagation of free radicals from one liposome to another.

Detailed Description Text (8):

A disadvantage of using saturated phospholipids when encapsulating unsaturated lipophilic materials is that the solubility of the lipophilic material to be encapsulated may be somewhat low. The low solubility thus limits the amount of the lipid that can be encapsulated. To overcome this lower loading capacity, small quantities of suitable additives such as glycerol are added to the lipid bilayer. Substantial increases in the amount of encapsulated lipid have been realized when glycerol is used in an amount corresponding to about 0.1 to 2 mole % of the phospholipid. Alternatively, the lipidic bilayer can be made up of a combination of suitable phospholipids or other liposome-forming lipidic components in which the unsaturated lipophilic materials are more readily soluble. The preferred lipidic materials employed in the bilayer are the phospholipids of choline such as egg phosphatidylcholine, dilauryloylphosphatidylcholine, dimyristoylphosphatidylcholine, dimyristoylphosphatidyl-choline, dipalmitoylphosphatidylcholine, distearoylphosphatidylcholine, 1-palmitoyl-2-myristoylphosphatidylcholine, 1-stearoyl-2-palmitoylphosphatidylcholine, 1-palmitoyl-2-stearoylphosphatidylcholine and dioleoylphosphatidylcholine.

Detailed Description Text (9):

In addition to the above phospholipids, a number of other lipids may be incorporated in the lipidic bilayer to reduce the permeability, strengthen the vesicle wall, and generally improve the physical characteristics of the liposome. Examples of such lipids may include sterols, cholesterol, aliphatic amines (such as long-chain aliphatic amines and carboxylic acids), long-chain sulfates and phosphates, dicetyl phosphate, butylated hydroxytoluene, tocopherol, retinol and isoprenoid compounds. The amounts will vary depending on the desired strength of the wall, the permeability of the encapsulated material through the wall, and the environment, but will generally not exceed a 1:1 ratio with the selected phospholipid.

Detailed Description Text (10):

When the liposomes are prepared for food use, it is desirable to reduce or eliminate the use of cholesterol as a stabilizer lipid. As an alternative, the saturated phosphatidylcholines having a chain length of 18 carbons and alpha-tocopherol have been found suitable for producing stable liposomes. The liposomes of the preferred embodiment of the invention have been found to be stable for the intended use, even in the absence of a stabilizer.

Detailed Description Text (11):

In addition, synthetic phospholipids containing either altered aliphatic portions, such as hydroxyl, branched carbon chains and cycloderivatives, ethers, amides, polyunsaturated derivatives, and halogenated derivatives, or altered hydrophobic portions containing carbohydrate, glycol, phosphate, phosphonate, quaternary amine, sulfate, sulfonate, carboxy, amine, sulfhydryl, and imidazole groups can be used to form liposomes in which the unsaturated lipophilic materials are more readily soluble. Several other phospholipids have been found useful in forming liposomes and can be employed depending on the material to be encapsulated and the environment in which they are to be used. By way of non-limiting examples, these phospholipids include phosphatidic acid, phosphatidylserine, phosphatidylethanolamine, spingolipids, phosphatidyl- glycerol, sphingomyelin, cardiolipin, glycolipids, gangliosides, cerebrosides, such as dilauryloyl phosphatidylglycerol, dipalmitoylphosphatidyl glycerol, distearoylphosphatidylglycerol, dioleoylphosphatidylglycerol, dimyristoylphosphatidic acid, dipalmitoyl phosphatidic acid, dimyristoylphosphatidylethanolamine, dipalmitoylphosphatidylethanolamine, dimyristoylphosphatidylserine, dipalmitoylphosphatidylserine, dipalmitoylsphingomyelin, and distearoylsphingomyelin.

Detailed Description Text (12):

In an alternative preferred embodiment of the invention, the phospholipid has a transition temperature slightly above temperatures required for processing the food product, such that the liposome will maintain its integrity. Depending on the final application and the type of food product, the liposome can have a transition

temperature slightly below body temperature. In this manner, the liposome may be solid at room temperature and fluid when consumed to increase the rate of release of the lipophilic component to provide proper taste and mouth feel. When fish oils are to be encapsulated, the phospholipid is selected such that the transition temperature is slightly above body temperature, whereby the liposomes will not dissolve or rupture in the mouth, but will rupture in the intestinal tract to release the fish oil. The transition temperature can further be controlled by the addition of suitable additives as are known in the art. When the liposome encapsulates a flavoring agent, it is desirable to have the phase transition temperature slightly below body temperature. When the food product incorporating the liposome is eaten, the liposome will rupture to release the flavoring agent. The shelf life of the liposome can be extended when the transition temperature is selected to withstand the anticipated storage conditions and expected temperature changes. Liposomes are generally considered to be solid at temperatures below the characteristic gel-liquid crystalline phase transition temperature of the phospholipid and fluid when above the transition temperature.

Detailed Description Text (13):

Although the preferred embodiment is directed to the encapsulation of fish oils, and in particular the omega-3 fatty acid-containing fish oils, the liposomes according to the invention may incorporate any substance which is soluble or miscible in the lipid bilayer. The oxidizable lipids which are common in the food industry and which may, by way of example, be incorporated in the lipid layer include eicosatetraenoic acid, eicosapentaenoic acid, docosahexaenoic acid, oleic acid, linoleic acid, linolenic acid, and arachidonic acids. The lipophilic material in the lipidic bilayer of the liposome is generally in the range of 10 to 30% by weight of the total lipids in the system. Other materials which may be dissolved in the lipidic bilayer of the liposome and are of particular interest include lipid-soluble vitamins, glycerol, acidulants, preservatives, flavorants, antioxidants, UV absorbers and tropicalizing agents for confectionery coatings and compounds.

Detailed Description Text (15):

A deodorized and refined menhaden fish oil having a high omega-3 acid content was selected to be dissolved in and carried by the lipid bilayer of a liposome formed from an unsaturated phospholipid. The liposome was prepared by dissolving 7.5 g of unsaturated soybean phosphatidylcholine (sold under the tradename Infusol) in 200 ml of reagent-grade chloroform containing 3.0 g of refined and deodorized menhaden fish oil in a 1 liter round-bottom flask at room temperature. To the flask, 66 ml of distilled water was added and mixed using a laboratory-scale homogenizer for about 2 minutes until a dispersion was formed. The dispersion was then placed in a rotary evaporator at about 40-50.degree. C. for about 40-60 minutes under vacuum to remove the solvent. Residual chloroform was removed by purging with nitrogen while shaking to break up the aggregates. The liposomes appeared as a tan-colored viscous gel. The resulting gel consisted of about 10% fish oil, 25% phospholipid, and 65% water by volume. The liposomes contained about 30% of encapsulated menhaden fish oil by weight of total lipid, which corresponds to about 10% of the total weight of the liposomes including residual water surrounding the liposome. Electron microscopy confirmed the presence of unilamellar and multilamellar liposomes. The liposome dispersion had substantially no fish odor or flavor.

Detailed Description Text (17):

A liposome preparation was made using a hydrogenated phosphatidylcholine sold under the tradename Phospholipon H-100. Phospholipon has a phase transition temperature of about 55.degree. C., which is higher than the Infusol. A solution of 7.5 g Phospholipon in 200 ml of chloroform was mixed with 3.0 g of refined menhaden fish oil. To the solution, 66 ml of distilled water and 0.68 g of glycerol were added while mixing using a laboratory-scale homogenizer. The dispersion was transferred to a rotary evaporator at about 60-63.degree. C. under vacuum to remove the chloroform. The resulting liposomes contained about 30% encapsulated menhaden fish oil by total weight of lipids present in the system. This corresponds to about 10% by weight of the liposome including residual water adhering to the outer surfaces of the liposome. The lipid particles were found to have a diameter generally ranging between 0.2 and 2.mu., with a few as large as 20.mu., as determined by laser light scattering. The smaller particles are believed to be unilamellar liposomes, while the larger particles, greater than 1.0.mu., are believed to be multilamellar liposomes.

Detailed Description Text (19):

A comparative study of the oxidative stability of the unencapsulated fish oil and the liposome-encapsulated fish oil prepared according to Examples I and II was made by measuring the peroxide value in milliequivalents per kilogram of lipid. The change in peroxide value provides an indication of the extent of oxidation of the lipids during storage. The control sample of menhaden fish oil and the liposome preparations were stored at about 4.degree. C. and brought to room temperature before determining the peroxide value using standard laboratory techniques. FIG. 1 shows the changes in the peroxide value over a period of 93 days for each sample. As can be seen, the control sample containing the unencapsulated fish oil demonstrated a rapid and substantial increase in the peroxide value, reaching a value of 37.5 mEq/Kg after 93 days. The liposome-encapsulated fish oil demonstrated a substantially lower peroxide value of 11 mEq/kg for Infusol-encapsulated fish oil and 8 mEq/kg for Phospholipon-encapsulated fish oil after essentially the same length of time. The data demonstrate a stabilizing effect on the liposome-encapsulated fish oil. The unencapsulated fish oil is shown to oxidize 3 and 4 times as fast as the encapsulated fish oil. In addition, the liposomes formed from the saturated phospholipid were shown to have a greater stabilizing effect than the liposomes from the unsaturated phospholipid.

Detailed Description Text (20):

In addition to the phospholipid, a sterol compound, such as cholesterol, a saturated phosphatidylcholine or alpha-tocopherol (vitamin E), can be added to stabilize the lipid wall of the liposome. The cholesterol has the effect of orienting the amphiphilic lipids to a more orderly crystalline array within the bimolecular layers, making the lipid layer much more resistant to diffusion of the encapsulated component. A suitable stabilizing amount is usually about 0.5% to about 3.0 % by weight of the phospholipid, but may also be in a mole ratio of 1:0.25 to 1:1, depending on the intended environment. When cholesterol is used, the ratio is generally quite small, such that the final amount of cholesterol present in the food product does not exceed the cholesterol level normally present. The liposomes prepared by the above method have been found to be sufficiently stable and do not require a stabilizer.

Detailed Description Text (21):

The liposomes containing the entrapped lipophilic material in the lipidic bilayer can be used in the form of an aqueous dispersion by adding the liposomes directly to the other ingredients in the formation of a food product. For example, in the preparation of a margarine, the liposomes containing encapsulated fish oil are mixed with the emulsion and molded by conventional means. The margarine emulsion will typically comprise 70 to 80% hydrogenated and interesterified vegetable oils, and an aqueous phase in the range of 20 to 30% containing water, proteinaceous materials such as skim milk powder, whey protein, casein, sodium caseinate, or soy protein, preservatives, emulsifiers, hydrophilic colloids, and flavorants. The liposomes in the preferred form of the invention are combined with the emulsion in amounts to produce a margarine having a final fish oil content of between 3 and 5% by weight of lipids in the system.

Detailed Description Text (22):

By way of a non-limiting example, a margarine composition is prepared by combining a hardened vegetable oil containing hydrogenated soybean oil and palm oil in a vessel and heating to slightly above the melting point. A suitable amount of lecithin, flavorants and colors are added to the fat portion. The water phase is added to the melted fat. The water phase contains salt, whey powder, potassium sorbate, citric acid alginate, and liposomes containing encapsulated fish oil as prepared in Example I. A sufficient amount of liposomes containing encapsulated fish oil is added such that the final margarine product has 3 to 5% by weight fish oil. The margarine emulsion is soft and readily spreadable and does not exhibit oil or water separation. The liposomes are able to withstand the shear stresses during mixing and heat treatment in the process. Since the liposomes are readily dispersible in the aqueous phase, the amount of fish oil to be added to the margarine composition is not limited by the characteristics of the liposomes, and fish oil can be added in any desired amount.

Detailed Description Text (23):

The novel liposomes, when prepared according to the present invention, can also be used as a means to control the release of various encapsulated components over time. For example, when the lipophilic material dissolved in the lipidic bilayer is a preservative, the liposome can be added to the food product as a dry powder or as a dispersion. By selecting the particular lipid of the lipid bilayer, the stability of the liposome wall can be controlled so that the wall breaks down at a reasonably predictable rate and at predetermined temperatures to release the preservative to the food product over an extended period of time.

Detailed Description Text (24):

Liposomes prepared from some phosphatidylcholines and other polar lipids may not be particularly stable to high shear. To improve the resistance of the liposomes to high shear, the phosphatidylcholine can contain an unsaturated fatty acid, which after liposome formation is covalently crosslinked by subjecting it to U.V. radiation. The exterior wall of the liposome is thus effectively polymerized to improve the shear stability of the liposomes.

Detailed Description Text (25):

Liposomes prepared from glycerophosphorycholine, bis-[12(methacryloyl)oxydodecanoyl]-L-.alpha.-phosphatidylcholine, or 1-palmitoyl-2-[12(methacryloyloxy)docecanoyl]-L-.alpha.-phosphatidylcholine are particularly suitable for cross-linking. Polymerization of the lipid bilayers, when formed from these phospholipids, is effectively carried out by direct U.V. irradiation at about 254 nm. Generally 1-3 hours is required to complete polymerization.

Detailed Description Text (26):

When the lipophilic material to be encapsulated is a highly unsaturated fatty acid, it is generally not desirable to subject the liposome to U.V. radiation, as this will tend to increase the rate of degradation of the unsaturated lipophilic component dissolved in the lipid bilayer. In this type of liposome, the shear stability can be improved by the addition of chemically reactive cross-linking agents such as glutaraldehyde or by the addition of stabilizing agents such as cholesterol or other polar phospholipids as are known in the art.

Detailed Description Text (27):

To further enhance the resistance to oxidation of the encapsulated material, the lipid layer and/or the aqueous layer may further include an antioxidant or oxygen scavenger. Typical antioxidants include ascorbic acid, ascorbyl palmitate, butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), EDTA, citric acid, propyl gallate, tocopherols, enzymes, and others as are well known in the art. As a further embodiment, the lipidic bilayer of the liposome may include a desired amount of a lipid capable of releasing a hydrogen ion to further stabilize the oxidizable unsaturated lipophilic material. The preferred lipids are those which contain a vicinal amino or vicinal hydroxyl group along the polar head region of the lipid molecule. For example, in the instance of vicinal amino groups, a glycolipid having a galactosamine or glycoamine residue is a suitable lipid. More commonly, the lipids will have a vicinal hydroxyl group at the polar head region. The preferred lipids include the glycolipids such as lactosyl ceramide, galactocerebroside, gangliosides, and trihexosylceramide, and phosphatidylinositol. When the lipid bilayer of the liposome is to include a lipid capable of releasing a hydrogen ion, it is preferred that the lipid be combined with the other lipids in a molar amount of about 10%. By including a suitable amount of the lipids in the lipidic bilayer, a source of donatable hydrogen ions is provided to the lipidic bilayer. It is generally understood that the ability to donate hydrogen ions provides a means of removing the active radicals from the autoxidation chain reaction.

Other Reference Publication (2):

"Accelerated ripening of cheese using liposome-encapsulated enzyme," International Journal of Food Science and Technology (1987), 22, 355-375.

Other Reference Publication (3):

"Procedure for preparation of liposome with large internal aqueous space and high capture by reverse-phase evaporation", Proceedings of the National Acad. of Science U.S.A., vol. 75; pp. 4194-4198 (1978).

CLAIMS:

1. A shelf-stable, edible liposome composition comprising liposomes having an oxidizable lipophilic material dispersed in the lipid layer of said liposomes providing said lipophilic material with an improved resistance to autoxidation, and wherein said liposomes comprise at least one entrapped aqueous material and at least one polar lipid bilayer, said lipid bilayer including an effective amount of a liposome-forming polar lipid having dispersed therein at least one edible oxidizable lipophilic material.
4. The composition of claim 3 wherein said lipophilic material is present in the amount of about 1-10% by total weight of the liposome composition.
8. The composition of claim 1 wherein the composition comprises a mixture of unilamellar and multilamellar liposomes.
9. The composition of claim 1 wherein the liposome further includes glycerol.
10. The composition of claim 9 wherein the liposomes further contain glycerol in an amount of about 0.1 to 2.0 mole % by weight glycerol of the phospholipid.
11. The composition of claim 1 wherein the aqueous layer of the liposomes includes an oxygen scavenger selected from the group consisting of enzymes and ascorbic acid.
13. A stabilized, oxidation-resistant fish oil containing composition comprising liposomes having at least one polar lipid bilayer, and at least one entrapped aqueous material, wherein said lipid bilayer comprises an effective amount of at least one liposome-forming polar lipid and at least one fish oil dispersed in said polar lipid whereby oxidation of said fish oil is inhibited.
16. The composition of claim 13 wherein the liposomes are a mixture of unilamellar and multi-lamellar vesicle liposomes.
17. The composition of claim 13 wherein the liposome has an average particle size of about 0.2-2.mu..
18. The composition of claim 13 wherein the liposome contains about 1-10% fish oil by total weight of the liposome.
22. The composition of claim 13 wherein said liposome further includes glycerol.
23. A shelf-stable, edible liposome composition containing liposomes having at least one polar lipid bilayer and at least one entrapped aqueous material; wherein said composition further contains about 10% to 30% of an oxidizable lipophilic material dispersed in the lipid layer of said liposomes to inhibit oxidation of the oxidizable lipophilic material, wherein the percent is by weight of the lipids in the liposomes.
24. A shelf-stable, edible liposome composition comprising liposomes having at least one lipid bilayer entrapping at least one aqueous material; the lipid bilayer including about 10 to 30% of an oxidizable unsaturated lipophilic material dispersed in a polar liposome-forming lipid; wherein said aqueous material includes at least one oxygen scavenging agent, and said oxidizable unsaturated lipophilic material is inhibited from oxidation.